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## Effect of 405nm High-Intensity Narrow-Spectrum Light on Osteoblast Function

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**INTRODUCTION:** A significant portion of medical devices fail due to acquired infection, with infection rates after arthroplasty surgery between 1-4%, and considerably higher after revision surgery. To reduce the associated costs of infection, a new preventative method is required. High intensity narrow spectrum (HINS) 405 nm light is a new technology shown to have bactericidal effects on a range of medically important bacteria[1]. The effect of HINS-light on osteoblasts and bacteria were investigated to determine the potential of this technology to contribute to infection prevention in operating theatres, during surgery and post-operative dressing changes.

**METHODS:** Osteoblasts were seeded at  $2 \times 10^4$  cells/cm<sup>2</sup> and allowed to adhere for 24 hr before being exposed to HINS-light at intensities from 0.8 to 15 mWcm<sup>-2</sup> for 1 hr ( $2.9 - 54$  Jcm<sup>-2</sup>). Expression of alkaline phosphatase (ALP) was measured at 24 and 72 hr post exposure and collagen synthesis was measured spectrophotometrically after staining with picric Sirius red. Expression of osteocalcin was assessed by ELISA at 24 and 72 hr post exposure. Cell morphology was assessed by SEM. The bactericidal effects of the maximum intensity which did not inhibit osteoblast function were investigated using a range of bacterial species. Bacteria were exposed in phosphate buffered saline (PBS) and on bacteriological agar (BA) plates, and surviving populations enumerated with results reported as % kill relative to control populations.

**RESULTS:** Low intensities of HINS-light (up to 5 mWcm<sup>-2</sup>) were shown to have no significant effect on osteoblast function. Expression of ALP, synthesis of collagen, and osteocalcin expression showed no significant decrease relative to control at any time point after 1hr exposure to HINS-light intensities at or below 5 mWcm<sup>-2</sup>. Exposure to 15 mWcm<sup>-2</sup> HINS-light for 1-hr caused a significant 46% decrease in ALP expression at 24hr post exposure, with no recovery occurring by 72hr. Osteocalcin expression was found to be

decreased by 56% at 72hr post exposure, and collagen synthesis by 35%. SEM of exposed osteoblasts showed obvious signs of damage to the cell membrane when exposed to higher intensities of 15 mWcm<sup>-2</sup> for 1-hr (Figure 1).

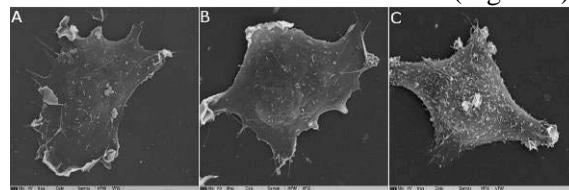


Fig 1: Effect of a 1-hr exposure to 5 and 15 mWcm<sup>-2</sup> (B and C) HINS-light on osteoblast morphology. Control (A). Scale bars are 5μm.

Following these results, 5 mWcm<sup>-2</sup> for 1-hr was determined as the maximum safe exposure for the osteoblasts and was applied to various bacterial species with varying results (Table 1).

Table 1. Inactivation (% kill) of bacteria exposed to 5 mWcm<sup>-2</sup> HINS-light for 1 hour.

	In PBS suspension	On agar surface
<i>S. epidermidis</i>	99.0 (±0.2)	40.0 (±22.2)
<i>S. aureus</i>	54.1 (±14.5)	21.6 (±2.8)
MRSA	47.8 (±14.6)	82.8 (±14.9)
<i>P. aeruginosa</i>	18.6 (±3.1)	76.2 (±13.4)
<i>A. baumannii</i>	12.3 (±2.3)	27.0 (±8.0)

**DISCUSSION & CONCLUSIONS:** One hour exposure to intensities of HINS-light of 5 mWcm<sup>-2</sup> and below have been shown to be safe for mammalian tissue. This dose has also been shown to have considerable bactericidal effects, suggesting that HINS-light has potential to be developed for safe use in the hospital environment for maintaining the sterility of surfaces and mammalian tissues.

**REFERENCES:** <sup>1</sup> M. Maclean et al. Applied and Environmental Microbiology 75(7), 1932-1937 (2009)

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